

WHAT IS CLAIMED:

1. A method for a large-scale production of antigen-specific intact antibody, said method comprising steps:
 - (a) isolating cDNA, mRNA or genomic DNA of genes for antibody light and heavy chains and assembling the antibody genes into expression cassettes containing the cDNA;
 - (b) preparing a recombinant *P. pastoris* yeast expression vector;
 - (c) constructing a recombinant *P. pastoris* yeast expression plasmid containing the expression cassettes of cDNA of the light and heavy chain genes encoding the antibody;
 - (d) cloning the antibody expression cassettes into the *P. pastoris* expression vector to generate recombinant plasmid;
 - (e) transforming *Saccharomyces cerevisiae* with the recombinant plasmid by placing said expression cassettes under the control of the AOX1 promoter fused to a *Saccharomyces cerevisiae* α -mating factor signal sequence;
 - (f) amplifying and isolating the recombinant plasmid;
 - (g) preparing and transforming *P. pastoris* with *Bgl*II, *Not*I, *Sac*I, *Sal*I or *Stu*I-linearized recombinant plasmid replacing the yeast chromosomal AOX1 sequence with AOX1-antibody gene cassettes of the recombinant plasmid;
 - (h) selectively growing the recombinants;
 - (i) screening yeast transformation colonies for (a) recombinant antibody expression;
 - (j) analyzing putative positive yeast clones for chromosomal integrates of the expression cassettes of heavy and light chain cDNAs;
 - (k) confirming the integrity of the DNA insert or junction sequence;
 - (l) inducing the recombinant antibody expression;
 - (m) confirming the intactness of the expression cassettes inserts with PCR and Northern blot analysis;
 - (n) detecting the presence of the recombinant antibody by Western blot; and

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(o) testing the recombinant antibody for specific antigen-antibody binding.

2. The method of claim 1 wherein the antibody genes are assembled into the expression cassettes by subcloning the antibody light and heavy chain cDNA in tandem *EcoRI*-*BglIII/BsmBI* fragments flanked by a *P. pastoris* signal sequence, preceded by a *P. pastoris* promoter at the 5'-terminus and a *P. pastoris* yeast transcription termination sequence at the 3'-terminus.

3. The method of claim 2 wherein the signal sequence is α -factor and wherein the promoter is AOX1-P.

4. The method of claim 3 wherein the yeast expression vector is pPICZ α .

5. The method of claim 4 wherein the yeast expression vector is prepared by restriction digestion with *EcoRI* and *BamHI*.

6. The method of claim 5 wherein the recombinant plasmid is pPICZ α LH.

7. The method of claim 6 wherein the recombinant expression plasmid pPICZ α LH is constructed by cloning the antibody genes expression cassettes into the *P. pastoris* expression vector.

8. The method of claim 7 wherein the replacement of the yeast chromosomal AOX1 with AOX1-antibody gene cassettes is by homologous recombination replacement.

9. The method of claim 8 wherein the selective growth of the recombinants is performed on a medium containing

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10. The method of claim 9 wherein the selective growth of the recombinants is performed on a medium containing g418, trimethoprin, or a compound that limits the growth of wild type *P. pastoris*.

11. The method of claim 10 wherein the screening of transformed colonies is by colony-immunoblotting.

12. The method of claim 11 wherein the screening is by PCR or by restriction analysis.

13. The method of claim 12 wherein the integrity of the DNA inserts or junction sequence is confirmed by nucleotide sequence analysis.

14. Intact antigen-specific antibodies produced by *P. pastoris* transformed with mouse, humanized mouse or human immunoglobulin genes, said antibody produced by the process comprising steps:

(a) isolating cDNA, mRNA or genomic DNA of genes for antibody light and heavy chains and assembling the antibody genes into expression cassettes containing the cDNA;

(b) preparing a recombinant *P. pastoris* yeast expression vector;

(c) constructing a recombinant *P. pastoris* yeast expression plasmid containing the expression cassettes of cDNA of the light and heavy chain genes encoding the antibody;

(d) cloning the antibody expression cassettes into the *P. pastoris* expression vector to generate recombinant plasmid;

(e) transforming *Saccharomyces cerevisiae* with the recombinant plasmid by placing said expression cassettes under the control of the AOX1 promoter fused to a *Saccharomyces cerevisiae* α -mating factor signal sequence;

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- (f) amplifying and isolating the recombinant plasmid;
(g) preparing and transforming *P. pastoris* with *Bgl*III, *Not*I, *Sac*I, *Sal*I or *Stu*I-linearized recombinant plasmid replacing the yeast chromosomal AOX1 sequence with AOX1-
5 antibody gene cassettes of the recombinant plasmid;
(h) selectively growing the recombinants;
(i) screening yeast transformation colonies for a recombinant antibody expression;
(j) analyzing putative positive yeast clones for
10 chromosomal integrates of the expression cassettes of heavy and light chain cDNAs;
(k) confirming the integrity of the DNA insert or junction sequence;
(l) inducing the recombinant antibody expression;
15 (m) confirming the intactness of the expression cassettes inserts with PCR and Northern blot analysis;
(n) detecting the presence of the recombinant antibody by Western blot; and
(o) testing the recombinant antibody for specific
20 antigen-antibody binding and intactness.

15. The antibody of claim 14 wherein the antibody genes are assembled into the expression cassettes by subcloning the antibody light and heavy chain cDNA in tandem *Eco*RI-
25 *Bgl*III/*Bsm*BI fragments flanked by a *P. pastoris* signal sequence, preceded by a *P. pastoris* promoter at the 5'-terminus and a *P. pastoris* yeast transcription termination sequence at the 3'-terminus.

30 16. The antibody of claim 15 produced by *P. pastoris* transformed with human immunoglobulin genes.

17. The antibody of claim 15 produced by *P. pastoris* transformed with humanized mouse immunoglobulin genes.

18. The antibody of claim 15 produced by *P. pastoris* transformed with mammalian or mouse immunoglobulin genes.

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5 19. A recombinant *P. pastoris* yeast expression vector containing dual expression cassettes, each carrying a cDNA copy of immunoglobulin light and heavy chain.

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10 20. An expression system comprising *P. pastoris* transformed with antibody genes for production of a recombinant antigen-specific intact antibody.

21. *P. pastoris* yeast transformed with expression cassettes carrying a cDNA copy of immunoglobulin heavy and light chain suitable for large-scale production of intact
15 antibodies.

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